

Quantitation of Free Metanephrines using VAMS-collected (Mitra®) blood samples

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Introduction

Metanephrine (MN), Normetanephrine (NMN) and 3-Methoxytyramine (3-MT), as stable metabolites of catecholamines are used as biomarkers of Pheochromocytomas (PCC) and Paragangliomas (PGL) tumors in clinical tests for diagnosis and treatment monitoring. Due to the risk of false positives, the patients should be supine, fasting, and at rest for at least 30 minutes before blood draw. In order to facilitate compliance with these restrictive conditions, Volumetric Absorptive Microsampling (VAMS) can be a helpful tool. The aim of the study was to adjust a ready-to-use kit (Chromsystems) for determination of Free Metanephrines (MN, NMN and 3-MT) in plasma for VAMS-collected (Mitra®) blood samples analysis.



30 µL plasma/whole blood → VAMS → SPE → LC-MS/MS



Table 1. Physiological plasma range for free methanephrines and level suggesting high probability of pheochromocytomas (PCC) and paragangliomas (PGL) based on literature data (Eisenhofer et al., 2019).

Analyte	Physiological plasma range ng/mL	Potential PCC/PGL level (≥3 × ULN*)
3-Methoxytyramine (3-MT)	0.0010–0.015	≥0.045
Metanephrine (MN)	0.055–0.077	≥0.231
Normetanephrine (NMN)	0.110–0.180	≥0.540

*ULN (Upper limit of normal)

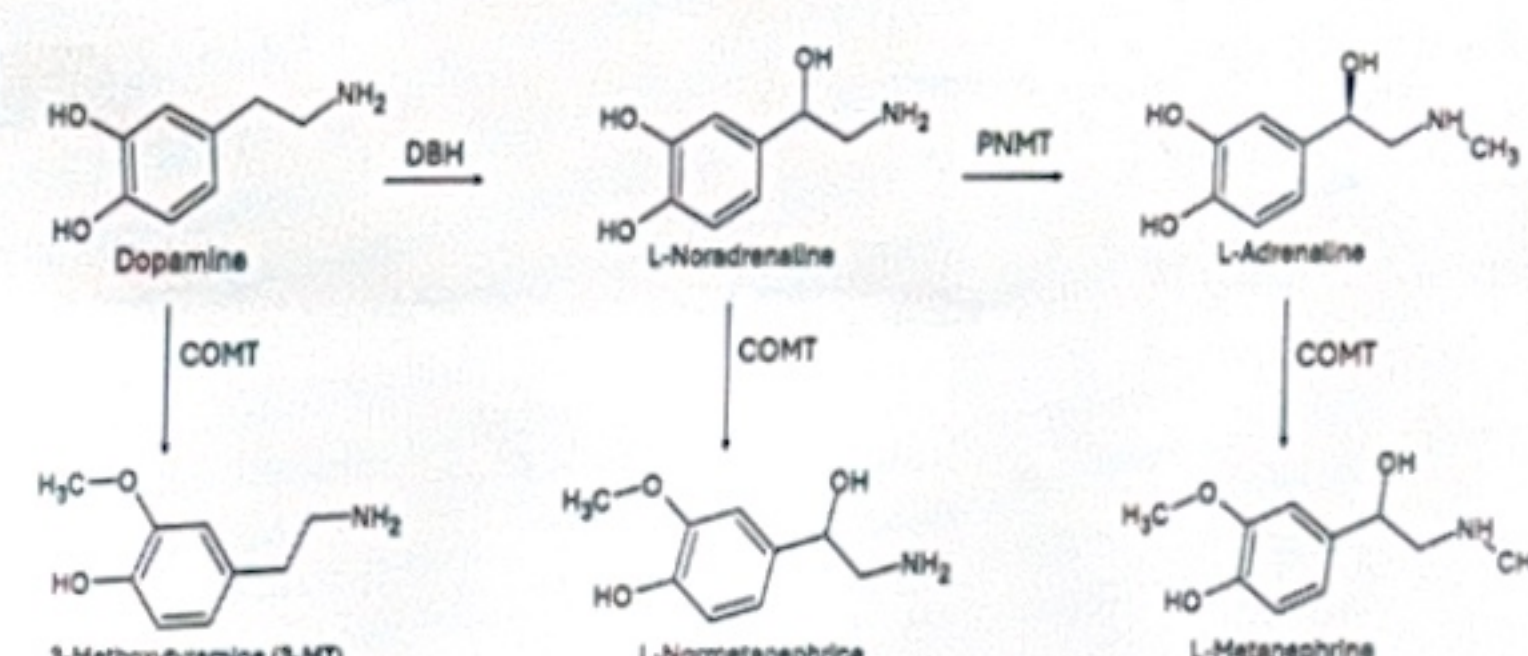


Figure 1. Metabolism of catecholamines to 3-methoxytyramine, L-normetanephrine and L-metanephrine [Chromsystems Instruction Manual. Free Metanephrines in plasma].

COMT (Catecholamine-O-Methyltransferase)
DBH (Dopamine β-hydroxylase)
PNMT (Phenylethanolamine N-methyltransferase)

Material and Methods

Blood/Plasma samples were prepared according to the procedure presented on the Figure 3. Analysis were performed on QTRAP 5500+ LC-MS/MS system (SCIEX).

Sample preparation



Figure 3. Sample preparation procedure.

Workflow

Sample preparation protocol optimization

Method verification Plasma MITRA device

Method verification Whole Blood MITRA device

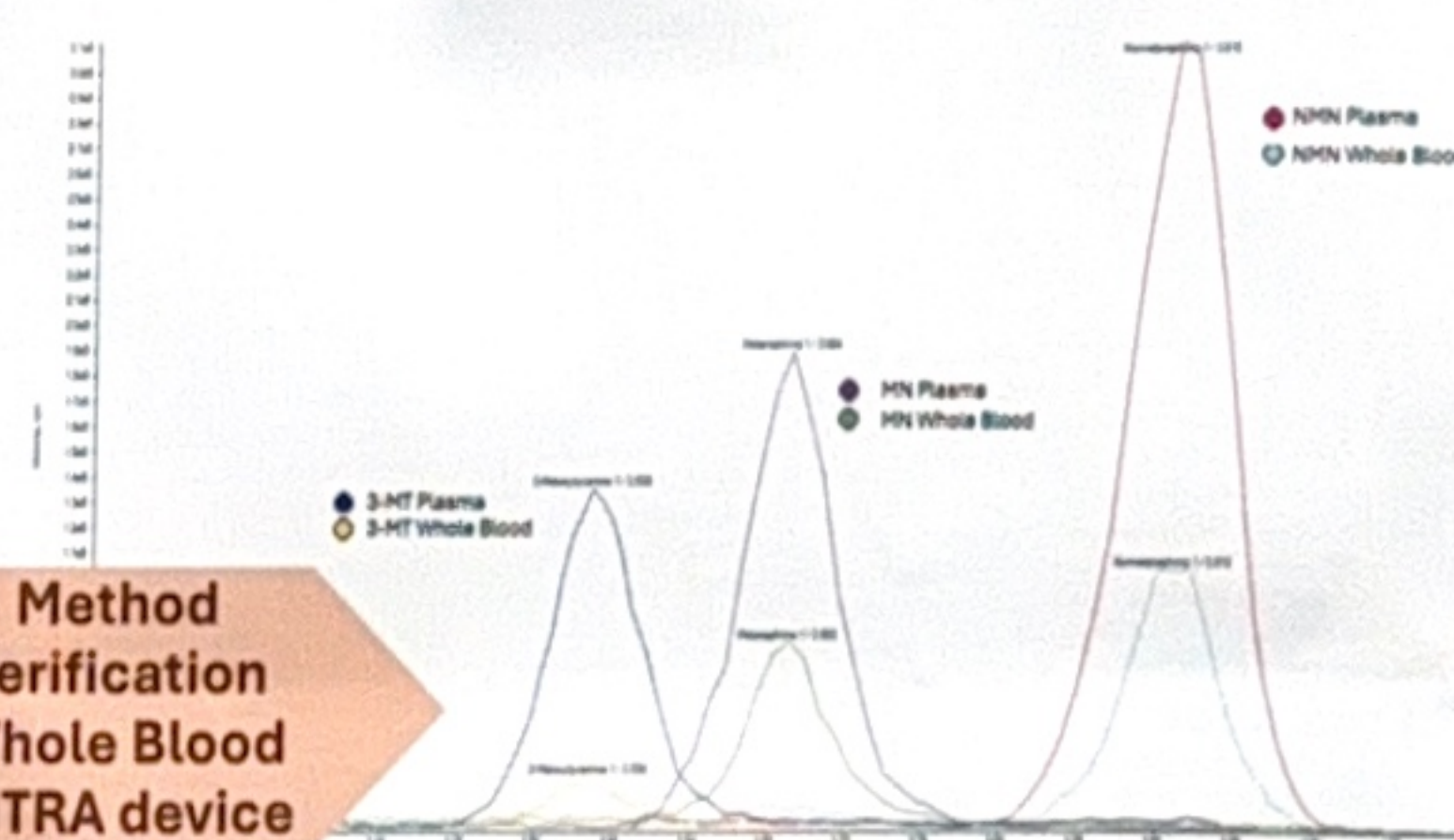


Figure 2. Chromatogram obtained for 3-methoxytyramine (3-MT), Metanephrine (MN) and Normetanephrine (NMN) for the same concentration level [CAL5] in plasma sample and whole blood sample prepared according to the same procedure.

Table 2. Preliminary validation results obtained in plasma and whole blood matrix for samples prepared using MITRA device

Analyte	Plasma MITRA LLOQ					Whole blood MITRA - expected LLOQ				
	C [ng/ml]	S/N ≥ 10	Accuracy [100±15%]	%CV [±15%]	Linearity	C [ng/ml]	S/N ≥ 10	Accuracy [100±15%]	%CV [±15%]	Linearity
3-Methoxytyramine (3-MT)	0.074	11	107.09	7.86	r = 0.998	0.074	10	89.13	> 15%	r = 0.987
Metanephrine (MN)	0.125	16	96.65	10.52	r = 0.998	0.125	17	98.67	7.21	r = 0.999
Normetanephrine (NMN)	0.181	23	99.11	13.00	r = 0.998	0.181	20	101.09	9.32	r = 0.999

Conclusions/Discussion

Strengths

- Only 30 µL blood sample
- Patient-friendly VAMS collection
- Minimal modification of Chromsystems protocol
- Successful adaptation for MN and NMN

Remaining challenge

- Whole-blood calibration for 3-MT
- Reduced response in VAMS whole blood
- Further optimisation required

3-MT: Matrix-dependent analytical challenge

- Lower response in whole blood (~8–10x)
- Low physiological concentration in blood
- Loss of linearity
- Non-reproducible LLOQ
- Endogenous signal in blank blood

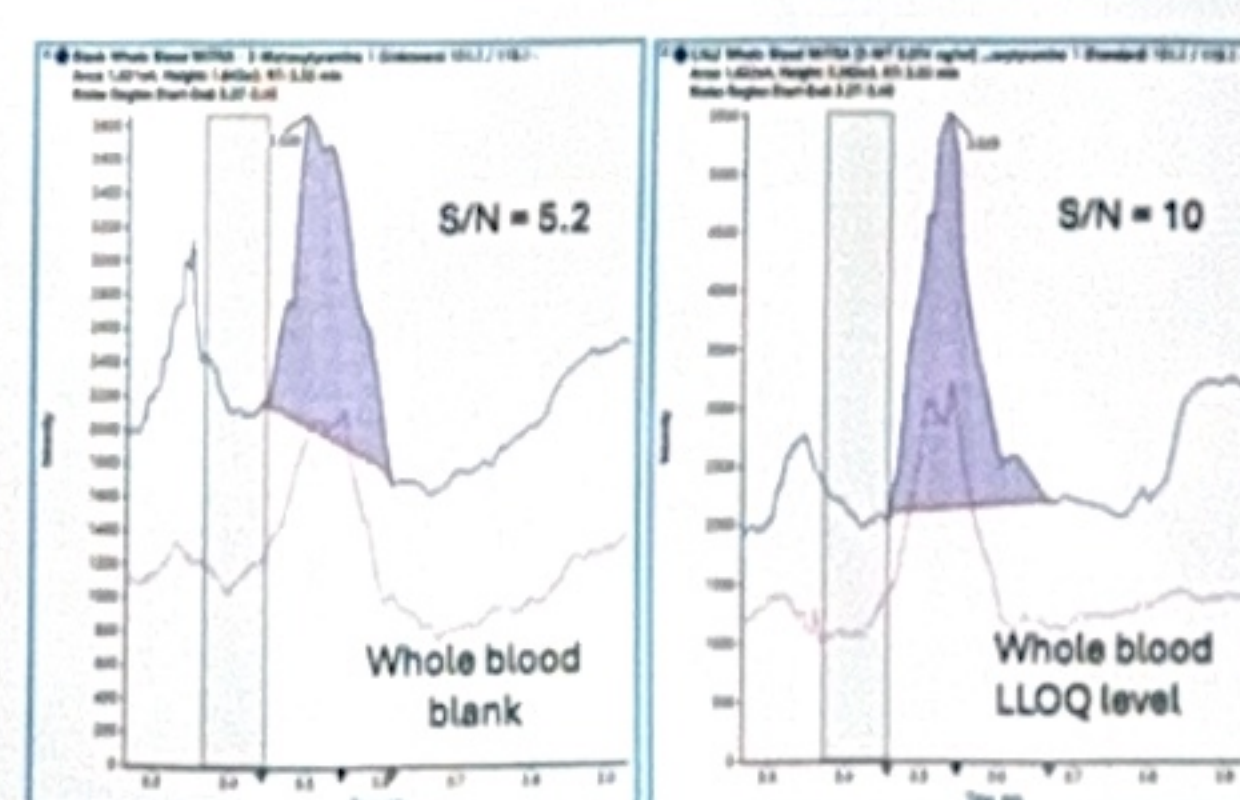


Figure 4. Chromatogram obtained for 3-methoxytyramine (3-MT) in whole blood blank sample (left) and calibrator 2 - LLOQ level (right) in whole blood collected on MITRA.